STRUCTURE AND FUNCTION IN THE PYLORIC CAECA OF ASTERIAS FORBESI

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In his book, *Comparative Physiology*, Scheer (1948, p. 352) makes the following statement concerning Echinodermata: "The process of digestion is not well known. Large diverticula are present in the asteroids. . . . Extracellular digestion is evident . . ., but the function of the extensive diverticula remains unsolved." A review of the literature on this subject reveals that this statement is largely justified. Although repeated attempts have been made to elucidate the mechanisms of digestion and absorption in asteroids, the conclusions drawn have been highly contradictory, and in many cases the experimental procedures are open to serious question. Indeed, it appears that no general agreement has been reached in descriptions of the histological details of the alimentary canal in starfishes.

The present report is a preliminary account of studies undertaken to clear up some of the existing confusion in the histology of the pyloric caeca, and to correlate by means of histochemical techniques the structural and functional aspects of the cells comprising the lining epithelium of these organs. Although no attempt has been made to present here an exhaustive review of all previous studies on the pyloric caeca, a brief analysis of the opinions of other investigators is included and an effort has been made to synthesize from all reliable sources a picture of the probable role of the pyloric caeca in alimentation.

Material and Methods

Animals used in these investigations were vigorous adult specimens of *Asterias forbesi*, ranging in size from 10 to 20 cm. in diameter. They were obtained by express from Woods Hole, maintained in 50-gallon marine aquaria, and fed regularly with portions of clam and mussel. For studies of the effect of nutritional state on the condition of the digestive diverticula, a series of animals was isolated and starved for varying periods ranging up to 8 weeks. Six to 8 weeks of starvation appeared to be the maximum which an animal of this size could withstand.

For use, one arm was removed from an animal and its paired pyloric caeca dissected out in sea water. Small pieces of the caeca were then taken and fixed in a series of fluids. These samples were subsequently processed and imbedded as appropriate for the various histological and histochemical techniques to be applied, as follows:

1) For general orientation, cell structure, and secretion granules, tissues were fixed in Zenker-formal, imbedded in paraffin by the dioxan method, and sec-

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tioned at 4 μ. These sections were stained in Mallory's phosphotungstic acid hematoxylin, Heidenhain's iron alum hematoxylin, Harris hematoxylin, or neutral gentian.

2) For demonstration of glycogen and related compounds, fixation was in Helly's fluid or in cold Rossmann, followed by treatment of paraffin sections by the Hotchkiss-McManus periodic acid-Schiff routine. Control slides exposed to the action of a buffered solution of malt diastase were used for differentiation between glycogen and other Schiff-positive substances.
3) For the recognition of mucin and other similar compounds (acid polysaccharides), paraffin sections of tissues fixed in buffered Orth's fluid or in basic lead acetate-formaldehyde were stained overnight in very dilute aqueous solutions of toluidine blue and dehydrated through 95% and absolute alcohol. This routine brings about a metachromatic staining of acid-polysaccharide elements. Representative sections of this material were also treated by the periodic acid-Schiff method.
4) For the localization, and, in part, the characterization of lipids, tissues were fixed in Baker's formal-calcium or formal-saline. For general recognition of lipids, tissues fixed in formal-saline, and soaked at 60° C. for 24 hours in 5% potassium dichromate, were imbedded in gelatin, sectioned on the freezing microtome, colored with Sudan black, and counterstained with Mayer's carmalum. For the localization of triglycerides, similarly fixed, postchromed, imbedded, and sectioned material was stained in 1% Nile blue A and differentiated in 1% acetic acid (Cain, 1947a). Phospholipid was investigated in material fixed in formal-calcium and treated by Baker's acid-hematein method, controlled by the pyridine-extraction test applied to adjacent samples fixed in weak Bouin's fluid (Baker, 1946).
5) For the demonstration of alkaline phosphatase activity, tissues were fixed in ice-cold acetone and imbedded in paraffin. Four-micron sections were incubated at 37° C. in a solution of sodium-beta-glycerophosphate and then carried through the routine of Gomori, in which sites of phosphatase activity are visualized as brown or black deposits of cobalt sulfide. Control sections were processed simultaneously, omitting only the substrate-incubation step.

It is a pleasure to acknowledge the capable technical assistance of Dorothy T. Clarke, Research Assistant in Biology at Brown University, in the conduct of a large part of this work.

Observations

A. General structure of the pyloric caecum

Each diverticulum constitutes one member of a pair in each arm, representing branches produced by the bifurcation of the single pyloric duct proceeding into the arm from an angle of the pyloric stomach. Each diverticulum is suspended by a pair of longitudinal mesenteries, enclosing between them a part of the epigastric coelom. The sheets comprising the mesenteries are continuous with the somatic mesothelium lining the body wall, and with the splanchnic mesothelium covering the diverticulum.

The pyloric duct continues the length of the caecum, forming a central tubular cavity known as Tiedemann's diverticulum. Serial outpocketings from the lateral walls of this tube form a row of pouches, whose cavities communicate broadly with that of the central passageway. By a series of vertical folds, the walls of these primary pockets form secondary bays, branching in a radial pattern. The secondary bays may also be subdivided, by both vertical and horizontal folds in their walls, to form the ultimate blind cavities of the caecum.

The wall of this complex organ is relatively simple, consisting of layers which may be described as follows:

a. As noted above, the organ is covered by a peritoneal layer. This is composed of small, cuboidal cells and is stretched thin over convexities but thrown into folds in markedly indented areas. Each cell of this layer bears a single, long flagellum.
b. Layers of muscular, nervous, and connective tissue fibers, variously represented, lie under the splanchnic peritoneum (for details, see Hamann, 1885, and Chadwick, 1923).
c. The epithelial lining is chiefly responsible for the thickness of the wall. It is composed of a single layer of extremely tall, slender cells forming a pseudostratified columnar epithelium, resting upon a basement membrane. This layer contains several distinct cell types, described below in detail. Speaking generally, the free ends of the epithelial cells are covered by a thick brush border, and each cell bears a single long flagellum. The height of the epithelium varies tremendously, being least in the angles of evaginated folds in the wall. At these points the lumen appears to extend almost to the basement membrane, while in the walls between these thin spots the epithelium may be from 75 to 90 μ in height.

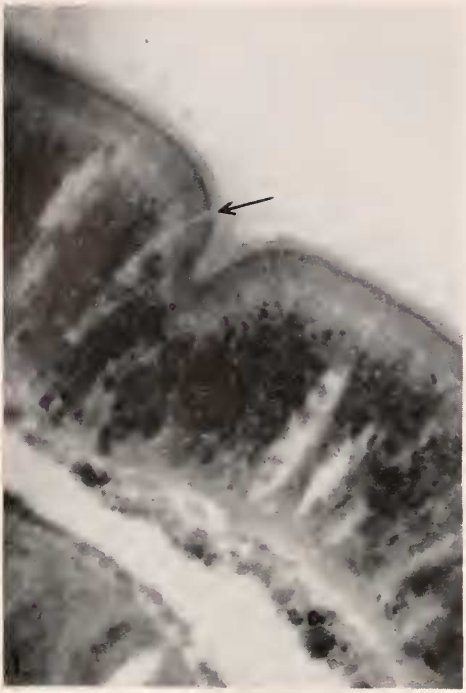
The following section will be concerned with elucidation of the cellular constituents of this lining epithelium and the characterization of the various cell-types represented, in terms of their histological and histochemical details.

B. Organization of the lining epithelium

The cells of which the epithelium is composed fall into the following categories: (a) special current-producers; (b) storage cells; (c) secretory or zymogen cells; (d) mucus gland cells.

a. Special current-producers are structurally highly adapted to the production of currents in the lumen of the diverticulum but apparently have no other special function. These cells are characteristic of the lining of Tiedemann's diverticulum and of the oral and aboral walls of the primary pockets arising from it. The structural details of these cells vary with their location, and apparently with the degree of crowding to which they are subjected. In the aboral wall of Tiedemann's diverticulum, between the attachments of the mesenteries, they are moderately tall, remarkably slender, and very closely packed (Fig. 9). In these cells the nuclei are long and spindle-shaped, measuring about 8 $\mu \times 2$ μ. They are granular, and each contains a small nucleolus. In other regions the current-producers are taller, less crowded, with densely-staining oval nuclei. In all, the nuclei lie at various depths in the epithelium, from a point near the basement membrane to within a short

PLATE I



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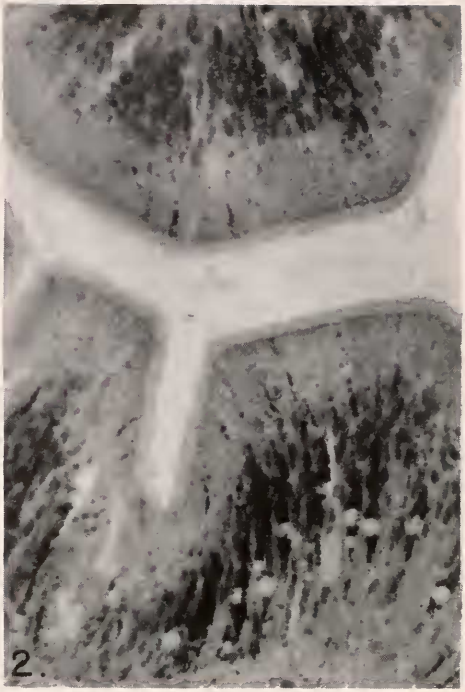

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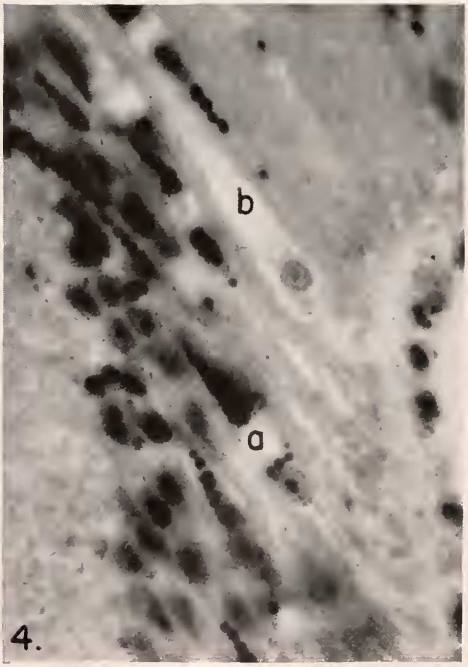

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distance of the free edge (Fig. 1). The cells are broadest in the nuclear region, and from the basal end of this area in each cell a tapering fibrous process extends to the basement membrane. Although this is difficult to ascertain, each cell appears to be represented at the surface of the lumen, where it bears a conspicuous brush border and a single long flagellum, ½ to ⅓ as long as the cell. The flagellum springs from a prominent blepharoplast lying just under the brush border and sending downward into the cytoplasm a stout chromophilic strand.

In all areas of which these cells are characteristic, mucus gland cells are abundant (Figs. 1 and 10).

b. Storage cells, in the lateral walls of the primary pockets and throughout the lining of the secondary bays and ultimate branches of the caeca, replace the current-producers as the most numerous class. These cells average about 5 μ in diameter and vary in height from 15 μ to a maximum of about 90 μ, depending upon their location and upon the size of the animal. Their nuclei are broadly oval or subspherical and generally stain densely and uniformly, except for the small nucleolus. The nuclei occupy a broad band limited approximately to the middle third of the cells. Each cell bears, again, a single flagellum originating in an apical blepharoplast and extending well beyond the brush border. Sections cut tangentially to the surface of the epithelium in these regions show that the cells are roughly polygonal in cross-section, and that the blepharoplasts, one in each cell, do not occupy the center of the apex but lie very near the cell membrane to one side (Fig. 6) and may be attached to it. Close study of sections passing longitudinally through this region gives the impression that the blepharoplast gives rise to one strand running in contact with the cell membrane and to another passing more directly downward into the cytoplasm (Fig. 3).

In a normal, well-fed animal, these cells contain abundant deposits of lipids, lying both above and below the nucleus in the form of moderate-sized droplets. The lipid droplets are larger and more numerous in the basal portions of the cells; above the nuclear region they become smaller and more sparse and are usually lacking from the distal quarter of the cell (Fig. 7). The Nile blue technique reveals that triglycerides predominate in the constitution of these lipid deposits; no elements have been recognized in the storage cells which are sudanophilic and stain other than pink with Nile blue. The acid hematein test has failed to reveal phospholipid deposits.

Figures 1 through 6 represent sections of material fixed in Helly's fluid, sectioned at 4 to 6 μ, and stained in Mallory's phosphotungstic acid hematoxylin. In all cases, the indicated magnification is approximately that of the figure as it appears here.

PLATE I

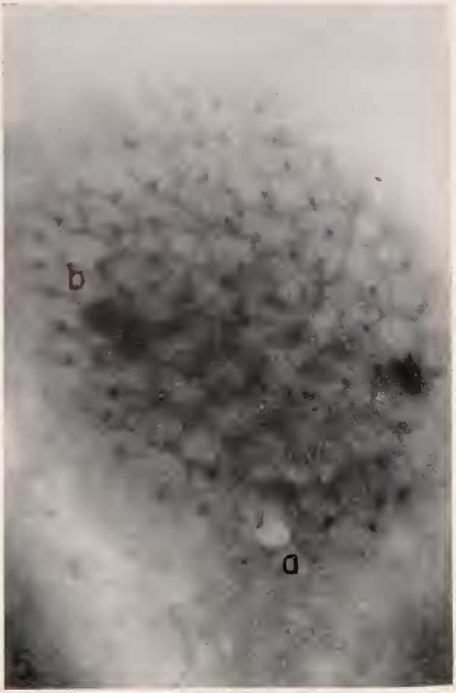
FIGURE 1. Special current-producing cells, moderately crowded region. Lumen above, perivisceral coelom below. Note row of blepharoplasts, brush border, flagella. Large clear areas are mucus goblets; mucus stoma indicated by arrow. 500 ×.

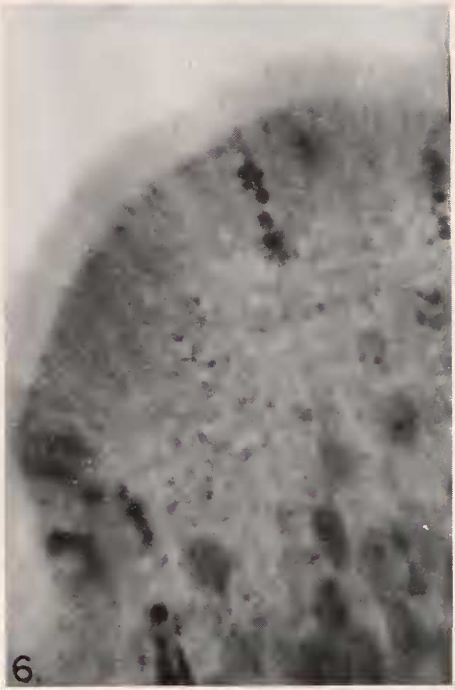
FIGURE 2. Storage and secretory cell areas, from same slide as Figure 1. Note vacuoles surrounded by secretory granules. 500 ×.

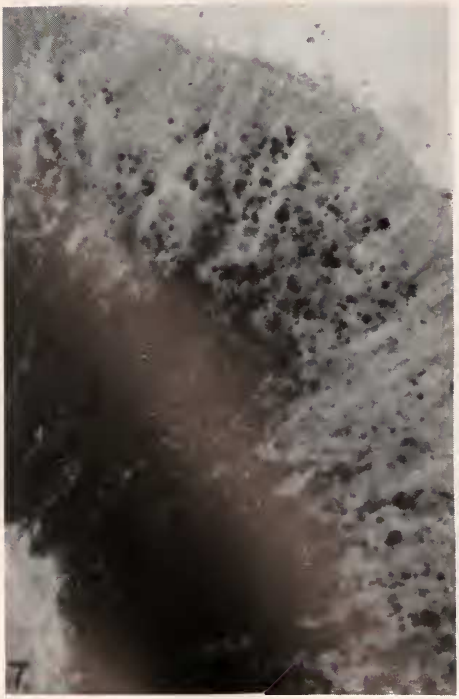
FIGURE 3. Part of same region, 1200 ×. Note extent of granule-train indicated by arrow; also divergent chromophilic strands from blepharoplasts.

FIGURE 4. Secretory cell region. At *a*, note relationship between nucleus and secretory products; at *b*, part of a mucus gland cell with empty mucus vacuole. Peritoneum at right; lumen off left. 1200 ×.

PLATE II







The ground-cytoplasm of the storage cells also contains a small amount of glycogen, in addition to more abundant stores of a related compound giving a positive reaction with the Hotchkiss-McManus technique even after prolonged exposure to saliva or malt diastase. This positive reaction is slowly abolished by the action of pancreatin. The substance does not stain metachromatically with toluidine blue. It is presumably a polysaccharide-protein complex. The small deposits of glycogen, and particularly the copious amounts of this unknown compound, have a distribution in the cell similar to that of the lipids; *i.e.,* they are most abundant in the region between nucleus and basement membrane, become scanty above the nucleus, and are absent from the distal ends of the cells (Fig. 9). Storage cells frequently contain large globules, giving the reactions of the unknown substance, scattered in the region above the nucleus (Fig. 9).

In an animal subjected to prolonged starvation, all of these reserves disappear from the storage cells. Glycogen, never abundant, is used first, and its disappearance is followed by a gradual decrease in demonstrable lipids (Fig. 8). The carbohydrate-protein compound also vanishes in the course of 6 to 8 weeks' starvation.

The greenish-yellow color of the pyloric caeca appears to be associated chiefly with the storage cells. Each of these cells contains numerous granules of a greenish pigment, limited to its distal portion. The pigment granules are not sudanophilic but frequently stain after prolonged treatment with phosphotungstic acid hematoxylin.

c. Secretory or zymogen cells are never encountered in the regions occupied by special current-producing cells but are numerous among the storage cells, which they resemble in some respects. Their nuclei are similar in size and shape to those of storage cells but have a more granular, less homogeneous appearance. The nucleus occupies the broadest region of the spindle-shaped cell, and in neighboring cells this swelling may be higher or lower, presenting a staggered arrangement of the nuclei in these closely-packed cells.

The outstanding feature of the secretory cells is their content of secretory granules, presumably zymogenic (Figs. 2, 3 and 4). These granules lie in clumps in the expanded region adjacent to the nucleus, both above and below the nucleus, and may extend in one or more rows completely to the free end of the cell. Very active cells also show dense aggregations of granules in their basal ends. Associated usually with the supranuclear granule clump appears a clear vacuole; very rarely, but occasionally, this vacuole may lie below the nucleus. The granules pass upward through the cell, crowding past the nucleus, and may often be observed as if on the

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PLATE II

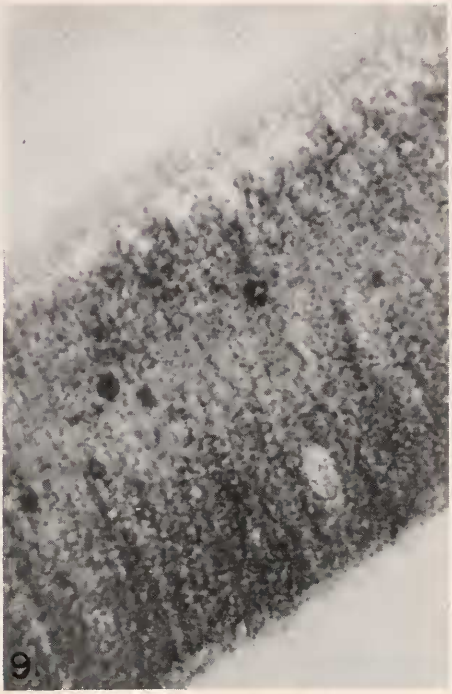
FIGURE 5. Distal ends of storage and secretory cells, section tangential to surface of epithelium. Note shape of cells, eccentric positions of blepharoplasts and their apparent attachment to cell membrane. At *a*, mucus stoma; at *b*, secretory cell. 1500 ×.

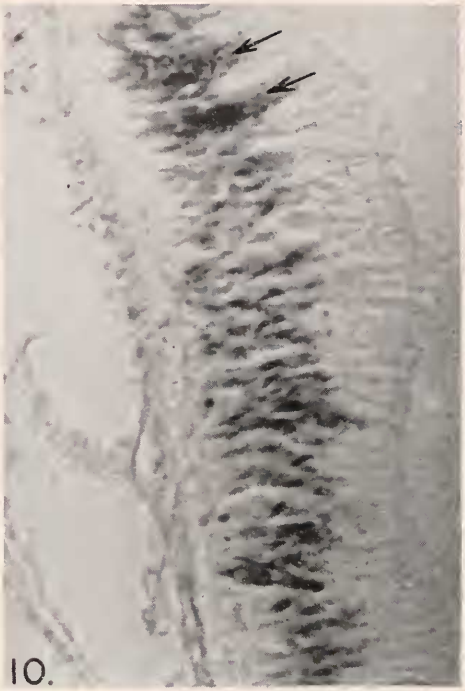
FIGURE 6. Distal ends of storage and secretory cells, longitudinal section. Note brush border, blepharoplasts, chromophilic strands, flagella. 1500 ×.

FIGURE 7. Storage cell area, showing lipid deposits. Note concentration of droplets toward basal ends of cells. Formal-saline; frozen section colored with Sudan black, counterstained with carmalum. 500 ×.

FIGURE 8. Storage cell area in animal after 8 weeks' starvation. Preparation exactly as in Figure 7; note exhaustion of stored lipids. 500 ×.

PLATE III





point of being extruded at the free end. The vacuole also appears to move upward through the cell with the granular clump but has not been observed near the apical end.

The vacuoles and secretory granules are well preserved by all effective cytoplasmic fixatives, and the granules are easily demonstrable by such staining methods as Heidenhain's iron alum hematoxylin, Mallory's phosphotungstic acid hematoxylin, and the neutral gentian technique. In frozen sections of material prepared for fat staining, they appear as clear, somewhat refractile spherules. Under the action of fixatives containing acetic acid they break down, and in sections of such material the secretory cells appear to contain only a rather densely granular, basophilic cytoplasm.

To date it has been impossible to establish the morphology of the distal ends of secretory cells. Unless it contains secretory granules the free end of a cell cannot with certainty be related to an underlying granule-filled nuclear region. The crowding of the cells is such that even in thin sections several storage and secretory cells are usually superimposed. In an unmistakable secretory-cell apex, the cytoplasm is so packed with granules as to obscure details (Fig. 5).

d. Mucus gland cells, as indicated above, are most numerous in regions lined by special current-producers. They also occur, less frequently, scattered among the storage and secretory cells. Mucus cells invariably extend almost to the basement membrane of the epithelium, and the nuclei of these cells are basally located. The overlying space is filled with mucus which usually expands the cell to a diameter many times that of the neighboring cells (Figs. 1 and 4). Above this expanded portion, at a variable distance from the base, the cell narrows and finally opens into the lumen of the caecum at a restricted stoma, devoid of any brush border or other structural specialization (Fig. 1). In fact, one may question whether the mucus cell as such comprises anything more than the basal portion of the gland; it is conceivable that the secretion is released from the gland cell at some lower point and exudes through a flask-shaped canal formed by the walls of adjacent cells.

In teased living preparations examined in sea water the secretion of the mucus glands consists of clumps of large globules. In fixed, sectioned preparations the globules are collapsed, and the secretion has the form of films or strands traversing the cavity filled in life by the globules. The material composing these strands reacts vigorously with the Schiff reagent after treatment with periodate and exhibits a strong, purplish metachromasia with dilute toluidine blue (Fig. 10). After its

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PLATE III

FIGURE 9. Storage cell area. Rossmann; Hotchkiss-McManus technique. Note basal concentration of Schiff-positive material, most of which is not removed by diastase; empty apical regions; large scattered globules. Coelom below, peritoneum missing. 500 ×.

FIGURE 10. Mucus gland cells in special current-producing area near mesentery attachment; *cf.* Figure 1. Note length of flagella (right), densely crowded cells. Arrows indicate two large metachromatically-staining mucus glands. Basic lead acetate-formaldehyde; dilute toluidine blue. 500 ×.

FIGURE 11. Storage cell area, alkaline phosphatase activity. Note concentration of activity at brush border and in apical areas; *cf.* Figures 10 and 12. Cold acetone; Gomori technique. The carmalum counterstain is responsible for most of the dark appearance of the lower areas. 500 ×.

FIGURE 12. Section adjacent to that shown in Figure 11, treated similarly but incubation with substrate omitted. Nuclei stained with carmalum. 500 ×.

release from the stomata, the secretion spreads thinly and generally over the free ends of the epithelial cells and by its characteristic reactions can be demonstrated about the fibers of the brush border.

In terms of the epithelium as a whole, alkaline phosphatase activity appears to be largely limited to the regions occupied by the storage cells. It is lacking or weak in the areas lined by the current-producing cells. This enzymatic activity is very strong at the free ends of the cells, centering about the region of the brush border and in the distal areas previously seen to be free of stored reserves (Fig. 11). It is weak or lacking in the deeper portions of the epithelium.

Discussion

It comes as a surprise to discover that both inner and outer epithelia of the alimentary canal are provided with vibratile organelles which from their length and distribution must properly be termed flagella, instead of cilia. Most previous investigators in describing these epithelia have referred to them as ciliated. For example, Hamann (1885) cites *"die heftige Wimperung"* of the caeca, and (p. 62) specifically likens the points of attachment of these organelles to those of the cilia on the molluscan gill. Van der Heyde in both the English (1922) and French (1923) versions of his dissertation speaks of cilia, as does Chadwick (1923). In justice to Hamann it should be stated that his observations of the current-producing organelles were largely accurate; he writes (p. 62) (here in connection with the cardiac stomach): *"Die Epithelzellen des Magendarmes sind lange cylindrisch bis haarfeine Zellen, welche je eine Wimper tragen."* Correct as to number, his use of the word *Wimper* is not in accordance with modern usage. On the other hand, Schneider (1902) describing the same elements uses the word *Geissel*, which in translation would be more likely to appear as "flagellum" than would *Wimper*. Authors concerned more with the physiology than with the morphology of Asterids have been unanimous in their use of the term "cilia" (Gemmill, 1915; Irving, 1924, 1926; Budington, 1942). While one does not wish to argue about words *qua* words, it appears only proper to bring the description of these structures in Asterias into line with consistent modern terminology.[2]

The histology of the digestive tract in various Asterids has been the subject of several investigations varying greatly in thoroughness, accuracy, and interpretation of facts. Hamann (1885) describes in some detail the mucus gland cells of the pyloric caeca, referring to them as *Becherzellen*. He concludes that they form originally at the base of the epithelium as small cells which move toward the free border, increasing in size and finally establishing stomata at the lumen border. The

[2] In this connection, Hyman (1940, footnote, p. 375) comments upon the failure of workers on Coelenterates to distinguish between cilia and flagella and is herself careful to make this distinction in every justifiable case. It should also be mentioned that upon closer study of developmental stages of *Asterias forbesi* in living and freshly-fixed material, as well as in thin paraffin sections, it is apparent that the general "ciliation" of the blastula and gastrula, and the frequently cited "ciliated bands" of the larva, are actually formed by cells bearing each a single, long, vigorous flagellum. The illustrations, and in part the text, of Korschelt and Heider (1895) appear to bear out this interpretation. If, as seems likely, flagellated rather than ciliated epithelia are the rule among Echinodermata, this peculiarity is shared among very few of the animal phyla (Porifera, Coelenterata in part, scattered instances in vertebrate tissues) and makes a close comparison of echinoderm larvae with the annelid-mollusk trochophore even more difficult.

remainder of the cell population of the epithelium he does not analyze, grouping them in a brief discussion simply as "epithelial cells," each with one or more *Wimpern* (*"das erste scheint die Regel zu sein"*), and containing a finely-granular cytoplasm. Considering the staining methods used by Hamann (acetocarmine, Böhmer's hematoxylin) it is not surprising that he found no trace of the secretory cells with their abundant coarse granules.

Schneider (1902) was apparently the first to observe these secretory cells. He terms them *Eiweisszellen* and describes their coarsely-granular secretion-product as moving up to the free ends of the cells in two or three rows. His description of the mucus gland cells is somewhat more accurate than Hamann's, and he recognizes also a third cell-type, *Nährzellen,* otherwise undifferentiated. It is significant that Schneider does not ascribe a storage function to any of these types and considers the pyloric caeca as organs of secretion and possibly absorption.

MacBride (1906) writes simply that the cells forming the walls of the pyloric sac and its appendages are tall, narrow, cylindrical cells crowded with granules which appear to be of the nature of digestive ferments.

Chadwick (1923) first established the regional differentiation of cell-types characteristic of the Asterid pyloric caeca. "At two points which are practically in the median line of each caecum," he writes (p. 22), "the structure of the wall differs from that which forms the lateral sacculi. One of these is that portion of the wall of the caecum which lies between the points of attachment of the two mesenteries by which it is suspended from the aboral wall of the ray, the other is a deep fold which traverses its free oral face. In the latter the nuclei of the epithelial cells are much more densely crowded than are those of the sacculi, and in sections they appear as a fairly broad band. These cells bear especially long and powerful cilia." Here Chadwick is clearly referring to the cells which I have termed "special current-producers." Chadwick also describes and figures the vacuoles in the secretory cells, mistaking them for small mucus glands; he does not mention the secretory granules which always surround them.

The latest attempt to establish the histological details of the pyloric caeca of *Asterias* was that of Dorman (1928). Alone among those who have worked with this tissue, Dorman describes the epithelium as consisting of several layers of cells, to which by certain histochemical tests he ascribes different functions. According to this description, "hepatic cells" lie in two or three rows near the basement membrane and are rich in fat and glycogen; "pancreatic cells" occupy the inner part of the wall and are described as "exceedingly granular" and said to contain some glycogen. The remaining cells are "interstitial cells," present in large numbers, some squamous and some spindle-shaped.

Aside from the fact that such an attempt to perpetuate the "hepato-pancreas" concept of invertebrate midgut diverticula seems unnecessary, this description is widely at variance with the results of the present study. However, the sources of Dorman's confusion are evident. The architecture of this epithelium is such that a section passing through it at an angle to the long axes of the tall columnar cells could easily be interpreted as showing several layers of cells; in such a section no single cell would actually appear in its entirety. The illusion of layering is heightened by the variation in the location of nuclei in these cells. Moreover, the stratification of the carbohydrate and lipid reserves in these tall cells, so as to fill their basal ends while leaving the apices relatively empty, would in a poorly-oriented

section lead to exactly the conclusion drawn by Dorman. Unfortunately, the single figure of this tissue furnished by Dorman is of no assistance in substantiating this explanation.

Several investigators have reported results of experimentation and observation relating to the functions of the pyloric caeca in Asterids. The question of their role in digestion and absorption was long debated, Frenzel (1892) maintaining that their sole function was to secrete enzymes which passed into the stomach and acted there upon ingested food. Frenzel convinced himself that no food ever enters the caeca, and in order to account for the fat deposits observed in the cells of the caeca was forced to postulate that these cells absorb from the coelomic fluid fats previously digested in the stomach. Opposite conclusions were reached by Chapeaux (1893), who found no absorption in the stomach and demonstrated (a) that carmine grains fed along with fibrin were subsequently found in the caeca, and (b) that oil-fed animals later showed fat-droplets stored in the cells of the caeca. The presence of a variety of digestive enzymes in the caeca was unquestioned, having been detected in extracts both by Chapeaux and by Fredericq (1878).

Stone (1897) and van der Heyde (1923) were unable to demonstrate detectable amounts of glycogen in the walls of the caeca by chemical analysis. This fact, together with a series of experiments involving perfusion of the digestive tract with iron saccharate, ammonium carminate, and olive oil, all of which were later found in the caecal epithelium, led van der Heyde to the conclusion (p. 139) that "this liver, without doubt, like the livers of so many other invertebrates, is principally and primarily an organ of absorption."

That more is involved in the function of the caeca than simple absorption of foods previously digested in the stomach is clearly indicated by the experiments of Irving (1926). In this work Irving perfused surviving isolated caeca of the starfish *Patiria miniata* with solutions of gelatin in sea water and was able to demonstrate a progressive rise in the non-protein nitrogen levels in the sea water in which these caeca were immersed. This technique, well-controlled, yielded unquestionable evidence that the caeca can digest proteins, and that the products of digestion are passed through the epithelia into the surrounding medium. In the intact animal, this medium would be the fluid of the perivisceral coelom, which is chiefly responsible for the distribution of absorbed nutrients throughout the body.

The work of Dorman (1928), apparently misguided in its morphological aspects, still indicates that reserves are stored in the cells of the caecal epithelium. This conclusion is justified also on the basis of the present investigation. Glycogen, another polysaccharide complex, and lipids are all demonstrable in the storage cells of the caeca. The conclusion that these represent stored reserves rests upon the evidence, presented above, that they disappear upon starvation. Starvation for one or two weeks is sufficient to demonstrate a detectable decrease in these reserves, and prolonged starvation results practically in their disappearance. This disappearance is not, moreover, the result of general moribundity of the tissue; sections of the pyloric caeca after 8 weeks of starvation showed a completely normal epithelium with no apparent decrease in the secretory activity of mucus gland and zymogen cells.

In connection with the oil-perfusion experiments of Chapeaux and of van der Heyde, which (together with the absence of glycogen) largely motivated van der Heyde's assumption of absorption as the primary function of the caeca, one looks

in vain in their accounts for any indication that they examined for fatty deposits the caeca of animals which had not been fed on oil. As indicated by the results of the present study, such a simple control procedure would undoubtedly have shown that the caecal epithelium of any normal animal in a reasonably good nutritional state contains abundant stores of lipid. Lacking such controls, their arguments lose force.

It is clear, however, that absorption must occur before reserves can be stored; the abundance of alkaline phosphatase activity at the free borders of the storage cells furnishes another indication of the importance of the pyloric caeca as organs of absorption.

From the work of Cain (1947b) and others, one would expect to find deposits of histochemically-detectable phospholipids in connection with the Golgi element and mitochondria of these epithelial cells. As indicated above, however, the acid-hematein test, controlled by the pyridine-extraction test, has given consistently negative results with this tissue. It should be pointed out that the classical cytology of the epithelial cells of the pyloric caeca has apparently never been described, and the Golgi element and mitochondria of these cells have not as yet been recognized.

In summary, considering the evidence from all sources, the functional role of the pyloric caeca appears manifold. Particulate, partially-digested food is drawn from the stomach along Tiedemann's diverticula by the action of the strong flagellated cells localized here. Side currents (see Irving, 1924, and Budington, 1942) carry the particles into the lateral branches and maintain a circulation; a variety of enzymes from the secretory cells completes the digestion of the food (for a recent account of the proteolytic enzymes involved see Sawano, 1936); the products of digestion are absorbed by the storage cells, where some are elaborated into reserves of polysaccharide and lipoid nature while others pass directly through the wall of the caecum into the perivisceral coelomic fluid for distribution.

All studies concerned with the enzymatic complement of the caeca agree that proteins and carbohydrates are digested here, but a question remains as to whether fats are actually digested, or whether they are strongly emulsified and pass into the cells as minute droplets. Chapeaux (1893) held that no lipase was present and postulated that fat digestion was a function of the free amoebocytes of the coelomic fluid, which engulfed and digested fat droplets passed unchanged through the wall of the pyloric caeca. This interpretation of the role of amoebocytes was disproved by van der Heyde (1923), without, however, any evidence that lipolytic activity occurs in the caeca. The fat-storing proclivities of the storage cells indicate that they handle large amounts of lipid, and an investigation of the mechanism involved would be of considerable interest.

The significance of the apparently two-fold secretory products of the zymogen cells is also unknown. Many invertebrates secrete digestive enzymes in the form of fluids enclosed in vacuoles and do not form zymogen granules. It remains an interesting possibility that the secretory cells of the Asterid pyloric caeca, known to elaborate a variety of enzymes, secrete their various products in different forms.

Summary and Conclusions

Histological and histochemical investigations of the pyloric caeca in *Asterias forbesi* reveal that their walls are composed of an outer splanchnic peritoneum made up of small, flagellated, cuboidal cells; layers of muscular, nervous, and connective

tissue fibers variously developed; and an inner epithelium generally composed of very tall, slender, flagellated cells bearing a dense brush border. Certain special areas (Tiedemann's diverticula) are lined with closely-packed cells termed "special current-producers," functioning to maintain directed movements of the contents of these central tubular cavities. Interspersed among these cells are numerous mucus gland cells. The walls of the lateral outpocketings of the central cavity are lined by an epithelium consisting of (a) secretory cells, producing conspicuous secretory (zymogen) granules and another product contained in small, clear vacuoles; and (b) storage cells, containing abundant lipids (almost entirely neutral fat), glycogen, and a polysaccharide-protein complex resistant to diastatic digestion. Mucus gland cells are less numerous in these areas. "Special current-producers" do not contain appreciable amounts of reserve substances, and the regions of the epithelium of which they form the lining do not show the vigorous alkaline phosphatase activity characteristic of the free border in the storage-cell areas. Starvation for 6 to 8 weeks results in a complete disappearance of all reserves but does not impair the secretory activities of mucus gland cells or of zymogen cells.

Previous works on the structure and functions of the Asterid pyloric caeca are summarized, and from all sources of evidence it is concluded that these organs function in digestion of food which passes into them from the stomach, in absorption of the products of digestion, and in storage of reserves. Transfer of nutrients to the coelomic fluid, for distribution throughout the body, is also a feature of their activities.

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